Effects of Chromium Picolinate and Ascorbic Acid Dietary Supplementation on Nitrogen and Mineral Excretion of Laying Hens Reared in a Low Ambient Temperature (7 ºC)

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Abstract

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The effects of chromium (chromium picolinate, CrPic) and ascorbic acid (L-ascorbic acid) supplementation on nitrogen (N), ash and mineral retention in laying hens (Hy-Line) reared under a low ambient temperature (7 ºC) was evaluated. One hundred and twenty laying hens (32-week-old) were divided into four groups, 30 hens per group. The laying hens were fed either a basal diet or the basal diet supplemented with either 400 µg of Cr/kg of diet, 250 mg of L-ascorbic acid /kg of diet, or 400 µg of Cr plus 250 mg of L-ascorbic acid/kg of diet. Retention of N, ash, Ca, P, Zn, Fe and Cr were highest with the combination of chromium and ascorbic acid and were lowest with the control diet ($P < 0.05$). Accordingly, excretion of N, ash Ca, P, Zn, Fe and Cr was lesser in chromium and ascorbic acid supplemented groups than the control ($P < 0.05$), that of combination of supplemental chromium and ascorbic acid being lowest. Results of the present study show that supplementing ascorbic acid and chromium, particularly as a combination, improved retention of mineral and decreased excretion of nitrogen, ash, Ca, P, Zn, Fe, and Cr in laying hens. Such a combination of supplementation can offer a potential protective management practice in preventing the detrimental effects of cold stress in laying hens. The results of the present study also show that chromium and vitamin C had additive effects on parameters measured at the present study.

C*old stress, chromium, ascorbic acid, mineral excretion, laying hen*

Low ambient temperature causes some adverse effects including increased feed intake, and decreased egg production, nutrient digestibility, and feed efficiency in poultry (Sagher 1975; Arad and Marder 1982; Ensminger et al. 1990; Spinu and Degen 1993; Sari 1993). Enviromental stress causes deficiencies in vitamin C (ascorbic acid) and chromium for poultry (McDowell 1989; NRC, 1997; Sahin and Sahin 2001). It has been reported that dietary ascorbic acid and chromium requirement in poultry was significantly affected by thermal stress and the negative effects of stress were prevented by ascorbic acid and chromium supplementation (McDowell 1989; Mowat 1994; NRC 1997; Sahin and Kucuk 2001; Sahin et al. 2001a; Sahin et al. 2002a). Dietary chromium supplementation has been shown to positively affect growth rate and feed efficiency of growing poultry (Cupo and Donaldson 1987; NRC 1997; Lien et al. 1999; Sahin et al. 2001b). These beneficial effects of Cr can be observed more efficiently under environmental, dietary, and hormonal stress (Anderson 1994; Wright et al. 1994). Supplemental dietary chromium is also recommended by NRC (1997) for animals undergoing environmental stress. Chromium stimulates and regulates the action of insulin (Anderson 1994; Mowat 1994) which is involved in anabolic processes (Colgan 1993). Also, through increasing the effectiveness of insulin, Cr indirectly potentiates

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ascorbic acid transportation (Mann and Newton 1975; Seaborn et al. 1994). In addition, chromium is thought to be essential for activating certain enzymes and for stabilizing proteins and nucleic acids (Anderson 1987; Linder 1991).

Ascorbic acid functions as a reducing agent and as an antioxidant. Previous studies have shown that ascorbic acid is an indispensable micronutrient required to maintain the physiological processes of certain animals including poultry (McDowell 1989). Poultry are known not to require a dietary source of vitamin C because of the ability of birds to synthesize its own. Pardue and Thaxton (1986) have documented evidence that particular environmental stressors can alter ascorbic acid utilization or synthesis in avian species. It has been also reported that ascorbic acid synthesis is inadequate under stress conditions such as low or high environmental temperatures, humidity, high production rates, and parasitic infestation (Sykes 1978; Hornig et al. 1984; McDowell 1989; Cheng et al. 1990). Several researches have documented a beneficial effect of ascorbic acid supplementation on growth rate and egg production in stressed-poultry (Thornton 1962; McDowell 1989; Bains 1996). At temperatures above or below thermoneutral zone, corticosteroid secretion increases in response to stress (Brown and Nestor 1973). By decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress such as cold stress-related depression in poultry performance (McDowell 1989). In evaluating the effects of supplemental chromium and ascorbic acid, nitrogen, ash and mineral excretion and retention have a substantial merit in understanding metabolic changes in cold-stressed poultry. Therefore, the objective of this study was to evaluate the effects of chromium and ascorbic acid supplementation on retention and excretion of nitrogen, ash and minerals in laying hens reared under a low ambient temperature (7 ^{o}C).

Materials and Methods

Animals

One hundred and twenty 32-week-old Hy-Line laying hens were obtained from a commercial company recognized by Ministry of Agriculture, Turkey. No animal losses were observed throughout the experiment. The experiment was in accordance with animal welfare, and was conducted under protocols approved by the Veterinary Control and Research Institute of Elazig-Turkey.

Dietary Treatments and Experimental Design

The laying hens were fed the basal diet or the basal diet supplemented with either 400 µg of Cr/kg of diet, 250 mg of L-ascorbic acid /kg of diet, or 400 µg of Cr plus 250 mg of L-ascorbic acid/kg of diet. Ascorbic acid (ROVIMIX® STAY-C®35) was specifically produced as a stabilized source of vitamin C for feed by a commercial company (Roche, Levent-Istanbul). Chromium Picolinate (CrPic, Chromax®, Prince Agri Products) was used as chromium source. Ingredients and chemical composition of the basal diet are shown in Table 1. The basal diet was a typical layer diet containing 11.6 MJ/kg and 17.6 % crude protein, and was calculated to meet or slightly exceed the nutrient requirements recommended by the National Research Council (1994).

The hens were randomly assigned to four groups, 30 hens each, according to their egg production which were similar among treatments. Water and the diets were offered ad libitum. The hen house was lit for 17 h per day. During the experiment, hen house’s temperature and humidity were measured four times a day (at 06.00, 12.00, 18.00, and 24.00 h). Average ambient relative humidity inside the hen house was 64 ± 7%. The mean value of daily temperature in the hen house was 7 ± 3 ^{o}C. Diets were fed for 15 wk between October15th and January 30th.

Retention and Excretion of Nitrogen and Minerals

At the end of experiment, 6 hens per treatment were individually caged to determine retention and excretion of dietary nutrients at the same low temperature. Nutrient retention was the amount retained per hen per day and was calculated based on feed consumption and the calculated analysis of the nutrient in the feed. Excreta of hens were collected for 3 days.

Laboratory Analyses

Chemical analysis of the diet and excrement samples were run using international procedures of AOAC (1990). Excrement N was chemically analyzed according to the method of Terpstra and de Hart (1974). To determine concentrations of Ca, Zn, Fe, and Cr, diet and excreta were dry-ashed (AOAC, 1990). Concentrations of Cr in diet and excrement samples were measured using atomic absorption spectrometer containing a graphite furnace and graphite tubes (Shimadzu AA-660-GFA-4B-P/N 204-03154-02). Calcium, Zn, and Fe concentrations were

Table 1
Ingredients and chemical composition of the basal diet fed to laying hens

Ingredients	%
Ground Corn	63.05
Soybean Mea	22.75
Wheat Bran	1.68
Animal Fat	1.50
Limestone	8.54
Dicalcium Phosphate	1.40
Vitamin Premix[a]	0.25
Mineral Premix[b]	0.20
DL-Methionine	0.20
Sodium Chloride	0.40
DM, %	91.10
Chemical Analyses (DM basis)	
ME, MJ/kg[c]	11.6
Crude Protein, %	17.6
Calcium, %	4.2
Phosphorus, %	0.8
Zinc, mg/kg	28
Iron, mg/kg	36
Chromium, μg/kg	1.1

[a]Mix supplied per 2 kg of diet: vitamin A, 15.500 IU; cholecalciferol, 2.500 IU; vitamin E, 15.5 IU; menadione, 2 mg; thiamin, 0.5 mg; riboflavin, 7 mg; d-pantothenic acid, 8 mg; pyrodixine, 2 mg; vitamin $B_{12,}$ 0.15 mg; folic acid, 1.5 mg; niacin, 30 mg.
[b]Mix supplied per 2 kg of diet: Mn, 80 mg; Fe, 60 mg; Zn, 50 mg; Cu, 7 mg; Iodine, 0.3 mg; Se, 0.15 mg; choline chloride, 400 mg.
[c]Calculated from the tabular values (NRC, 1994).

measured at specific wavelengths for each element by using atomic absorption spectrometer. Calibrations for the mineral assays were conducted with a series of mixtures containing graded concentrations of standard solutions of each element.

Statistical Analyses

All data were analyzed by one-way ANOVA (GLM procedure of SAS; SAS Institute, 1996) to test for the effects of the dietary treatments. When a significant treatment effect was observed, a Duncan's new multiple range test was used to compare means. Treatment effects were considered with the significant level at $P < 0.05$. Experimental unit was individual bird.

Results

The effects of supplemental dietary chromium and ascorbic acid on excretion and retention of nitrogen, ash and mineral in laying hens when exposed to a cold environment are shown in Table 2. A combination of chromium and ascorbic acid, rather than each separately, provided the greater nitrogen and mineral retention and lower excretion rates in laying hens exposed to a cold environment compared to the control group. Retention of N, ash, Ca, P, Zn, Fe and Cr were highest with the combination of chromium and ascorbic acid and were lowest with the control diet ($P < 0.05$). Accordingly, excretion of N, ash, Ca, P, Zn, Fe and Cr was lesser in the chromium and ascorbic acid supplemented groups than the control ($P < 0.05$), that of a combination of supplemental chromium and ascorbic acid being the lowest.

Discussion

It is known that poultry performance and nutrient digestibility decrease when ambient temperature goes below the thermo-neutral zone (Arad and Marder 1982; Ensminger et al. 1990; Sari 1993). At such temperatures, corticosteroid secretion increases in

Table 2
The effects of supplemental chromium and ascorbic acid on the nitrogen and minerals retention and excretion of laying hens reared at a low ambient temperature (n=6)

Item	Treatments*				
	C	Cr	Vit C	Cr+Vit C	SEM
Retention					
Nitrogen, (g/hen/d, DM)	1.9[c]	2.4[b]	2.3[b]	2.7[a]	0.09
Ash, (g/hen/d, DM)	6[c]	9[b]	9[b]	13[a]	0.5
Calcium, (g/hen/d, DM)	2 [c]	3[b]	3 [b]	4 [a]	0.02
Phosphorus, (g/hen/d, DM)	0.2 [c]	0.3[b]	0.3[b]	0.4[a]	0.005
Zinc, (mg/hen/d, DM)	1 [c]	2 [b]	2 [b]	3 [a]	0.2
Iron, (mg/hen/d, DM)	10[c]	13 [b]	14[b]	17 [a]	1
Chromium, (mg/hen/d,DM)	0.05[c]	0.07[b]	0.07[b]	0.08[a]	0.001
Excretion					
Nitrogen (g/hen/d, DM)	1.6 [a]	1.5 [b]	1.5[b]	1.3[c]	0.003
Ash, (g/hen/d, DM)	10 [a]	9 [b]	9[b]	8 [c]	0.2
Calcium, (g/hen/d, DM)	2.5 [a]	2.4 [b]	2.4[b]	2.3[c]	0.05
Phosphorus, (g/hen/d, DM)	0.6[a]	0.5[b]	0.5 [b]	0.4 [c]	0.002
Zinc, (mg/hen/d, DM)	11[a]	10[b]	10 [b]	8[c]	0.03
Iron, (mg/hen/d, DM)	36 [a]	33[b]	32 [b]	30 [c]	0.9
Chromium, (µg/hen/d,DM)	0.09[a]	0.07[b]	0.07[b]	0.06[c]	0.005

[a,b,cd]: Mean values within a row with no common superscript differ significantly ($P < 0.05$).
*C: control (basal) diet, Cr: control diet +400 µg of Cr/kg , Vit C: control diet +250 mg of L-ascorbic acid /kg of diet, Cr+Vit C: control diet + 400 µg of Cr/kg + 250 mg of L-ascorbic acid/kg.

response to stress (Brown and Nestor 1973). Kutlu and Forbes (1993) reported that ascorbic acid reduces the synthesis of corticosteroid hormones in birds. By decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of environmental stress such as cold stress-related depression in poultry performance (McDowell 1989; Pardue et al.1985). Environmental stress has been shown to increase mineral excretion (Smith and Teeter 1987). El-Husseiny and Creger (1981) found that broilers reared under environmental stress had lower rates of Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn retention. Stress increases chromium mobilization from tissues and its excretion and also depresses ascorbic acid synthesis (Sykes 1978; Hornig et al. 1984; Borel at al. 1984; Pardue and Thaxton 1986; Anderson 1987; McDowell 1989); thus stress may exacerbate a marginal chromium and ascorbic acid deficiency or an increased chromium and ascorbic acid requirement, implying that both chromium and ascorbic acid should be supplemented as shown in the present study. Wallis and Balnave (1984) found that the digestibility of amino acids was decreased by a high environmental temperature in broilers. Similarly, Zuprizal et al. (1993) have shown that true digestibility of protein and amino acids decreased as the temperature increased from 21 to 32 ^{o}C. The results of the current metabolic trial showed that retention of nitrogen and Ca, P, Zn, Fe as well as Cr, is improved and excretion, decreased by supplemental chromium and ascorbic acid. We cannot explain the decrease observed in chromium excretion in chromium supplemented groups (Table 2). A chromium supplementation at a higher level could increase the excretion of chromium. Since ascorbic acid and chromium (postulated to be antioxidants) have a protective effect on pancreatic tissue against oxidative damage (McDowell 1989; Preuss et al. 1997), they may help pancreas to function properly including secretions of digestive enzymes, thus improving retention of nitrogen and minerals. In a previous a study we found that ascorbic acid supplementation reduced serum concentration of malonaldehyde (MAL), an indicator of lipid peroxidation, in stressed hens (Sahin et al. 2002b). An increase in retention of nutrients in laying hens in the present study could have been due to such positive effects of ascorbic acid and/or chromium. It has also been reported that in stressed-mice, loss of zinc, copper, iron and manganese was reduced by supplemental chromium (Schrauzer et al. 1986). Sahin and Sahin (2001) reported a decrease in utilization of dry matter, crude protein, and ether extract in laying hens kept under low ambient temperature (6.2 °C) and that supplemental chromium and vitamin C alleviated these negative values to a higher extent than control group. Sahin and Kücük (2001) reported that digestibility of nutrients increased with dietary vitamin C in Japanese quail under stress conditions.

In the present study, the magnitude of the increases or decreases in retention and excretion of nitrogen, ash and minerals was greater when a combination of ascorbic acid and chromium was supplemented than when supplemented separately. These results revealed additive effects of ascorbic acid and chromium, indicating that ascorbic acid and chromium work together or act synergistically. Similarly, Carol et al. (1994) found an interaction between Cr and vitamin C on bone and brain Mn retention and distribution in guinea pigs, and stated that dietary Cr may influence ascorbic acid metabolism via protecting ascorbate from oxidative destruction. In addition, insulin is known to play a role in ascorbic acid transportation in red blood cells, and glucose competitively inhibits ascorbic acid transport (Mann and Newton 1975). Through increasing the effectiveness of insulin, chromium indirectly promotes the ascorbic acid transportation (Seaborn et al. 1994).

Results of the present study show that supplementing ascorbic acid and chromium, particularly as a combination, improved retention of mineral and decreased excretion of nitrogen, ash, Ca, P, Zn, Fe, and Cr in laying hens. Such a combination of supplementation can offer a potential protective management practice in preventing the detrimental effects of cold stress on laying hens.

Vliv doplnění diety chrómpikolinátem a kyselinou askorbovou na exkreci dusíku a minerálních látek nosnicemi odchovávanými při nízké teplotě prostředí (7 °C)

V práci byl hodnocen účinek doplnění diety chrómem (chrómpikolinát, CrPic) a kyselinou askorbovou (L-askorbová kyselina) na retenci dusíku (N), popelovin a minerálních látek u nosnic (linie HY) odchovávaných při nízké teplotě okolního prostředí (7 °C). Skupina 120 nosnic (stáří 32 týdnů) byla rozdělena do 4 skupin, po 30 slepicích v jedné skupině. Nosnice byly krmeny jednak základní dietou, jednak základní dietou doplněnou 400 μg Cr/kg krmiva nebo 250 mg L – askorbové kyseliny/kg krmiva anebo 400 μg Cr a současně 250 mg L-askorbové kyseliny/kg krmiva.

Nejvyšší retence N, popelovin, Ca, P, Zn, Fe a Cr byla zaznamenána u kombinace chrómu a kyseliny askorbové a nejnižší byla u kontrolní diety ($P < 0.05$). Podobně tomu bylo i u exkrece N, popelovin, Ca, P, Zn, Fe a Cr: nejnižší byla u skupin krmených s doplňkem chrómu a kyselinou askorbovou ve srovnání s kontrolní skupinou ($P < 0.05$). Z výsledků studie je zřejmé, že doplnění výživy kyselinou askorbovou a chrómem, zvláště v kombinaci obou, zlepšuje retenci minerálních látek a snižuje sekreci N, popelovin, Ca, P, Zn, Fe a Cr u nosnic. Uvedená kombinace jako krmný doplněk by mohla mít případný protektivní účinek při prevenci škodlivých následků chladového stresu u nosnic. Výsledky této studie rovněž dokládají, že chróm a vitamín C měly aditivní účinek na sledované ukazatele.

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